Penn iGEM Transformation Protocol

Preparation:

Combine 10 tubes (3.5 mL each) of aerobically grown bacteria into a 50 mL falcon tube. Count cells and determine concentration. Must be at magnitude of at least 10^7cells/mL to proceed. Centrifuge at 3700 RPM for 15 minutes. Pour off supernatant. If supernatant is murky, spin for longer. Resuspend pellet with 25 mL of 10 mM TES buffer containing 272 mM sucrose (pH 7.5). Centrifuge at 3700 RPM for 10 minutes. Resuspend pellet in total volume of TES + sucrose buffer needed to concentrate cells to 10^9 cells/mL. Split into 50 µL aliquots. Store at -80oC.

Electroporation:

Place 0.1-cm electroporation cuvette on ice. Add 1.5 µL of DNA to 50 µL aliquot of competent cells. Stir gently with pipet. Transfer solution to electroporation cuvette. Add 500 µL TES + sucrose buffer to an Eppendorf tube as recovery media. Electroporate with a Gene Pulser® (Bio-Rad Laboratories, Richmond, Calif.) at following settings: Capacitance 25 µF // resistance 200 Ω // 1mm cuvette // 10 kV/cm (1000V). Transfer 55 µL of electroporated solution into Eppendorf tube with recovery media. Recover at 30oC in a shaker at 100 RPM for 16 hours before plating.